Reduction of Nitrate in a Meat System by *Lactobacillus plantarum*

J. L. SMITH AND S. A. PALUMBO

Eastern Regional Research Center, Philadelphia, Pennsylvania 19118, U.S.A.

1. *Lactobacillus plantarum* in commercial lactic acid starter cultures used to ferment meat products reduced nitrate to nitrite in sterile meat. The nitrite reacted with the meat myoglobin to yield nitrosylmyoglobin.

MANUFACTURERS of fermented sausage depended traditionally on the reduction of nitrate for curing. The production of nitrite from nitrate in fermented sausage was due primarily to the acid-sensitive micrococci (Niinivaara 1955; Deibel & Evans 1957). As such organisms would be inhibited by rapid acid production by lactic acid bacteria, the manufacturer of Lactacel starter cultures recommends that nitrite ($NaNO_2$, 77 parts/10^6) be added to the meat (Deibel & Evans 1957; Anon. 1973*a*,*b*).

When Zaika *et al.* (1976) used Lactacel MC starter culture and nitrate (100–1800 parts/10^6) to produce Lebanon bologna, they obtained an excellent cured-meat colour. Costilow & Humphreys (1955) have shown that certain strains of *Lactobacillus plantarum* can reduce nitrate to nitrite when growing in nutrient media. As Lactacel MC starter culture contains *L. plantarum* and *Pediococcus acidilactici*, it was thought possible that the starter culture may reduce sufficient nitrate to produce the cured-meat colour in sausages. No one seems to have considered the possibility that lactic acid bacteria might reduce nitrate to nitrite during the fermentation of sausages. This neglect may be due in part to the fact that nitrate reduction is not considered to be an important feature in the characterization of lactic acid bacteria (Sharpe *et al.* 1966). The reduction of nitrate by *L. plantarum* in minced meat and by other lactic acid bacteria in a nutrient medium is described in this paper.

Materials and Methods

Lactic acid starter cultures

Frozen concentrates of starter cultures were obtained from Merck and Co. (Rahway, N.J.); Lactacel contained *P. acidilactici*, Lactacel DS *L. plantarum*, and Lactacel MC *P. acidilactici* and *L. plantarum*. To prepare the inoculum (*ca.* 5×10^9 viable cells/ml) for meat, 1 volume of frozen culture and 3 volumes of sterile distilled water were mixed for a few seconds in a sterile blendor.

The lactic acid bacteria used to study nitrate reduction in nutrient medium were obtained from our Dairy Laboratory stock culture collection. The organisms were grown in APT Broth (Difco) for 24 h at 35 °C.

Ground beef chuck (100 g) in blendor jars (500 ml) was autoclaved (20 min/121 °C). One ml of the diluted starter culture and 25 ml of a filter-sterilized water containing NaCl 2 g, glucose 1 g, L-cysteine 50 mg, and $NaNO_3$ 100 mg or $NaNO_2$ 10 mg were added to the cooled meat. The meat and solutions were mixed until a smooth slurry was obtained and this was incubated at 35 °C for 24 h.

A modification of the procedure of Hornsey (1956) was used to measure cured-meat colour; extraction and determination of the nitrosyl pigment was done rapidly in partial darkness to minimize destruction of the pigment. After 24 h incubation, meat (10 g) and sodium bicarbonate (0·5 g) were ground for *ca.* 1 min and the paste mixed thoroughly with acetone (40 ml). This mixture was centrifuged (1 min; 12 000 **g**; 2 °C) and the absorbance (at 540 nm) of the supernatant determined with a Beckman Model B Spectrophotometer.

Nitrate reduction in nutrient medium

Diluted starter culture or lactic acid bacteria were inoculated into Tryptic Nitrate Medium (Difco) at 35 °C and tested (Anon. 1957) daily for nitrite.

Results and Discussion

Nitrate was reduced to nitrite in nutrient medium by 4 of 6 strains of *L. plantarum*, by 3 species of *Streptococcus*, 3 species of *Lactobacillus* and 2 species of *Leuconostoc.* Lactacel MC and DS which contained *L. plantarum* reduced nitrate whereas Lactacel which contained *P. acidilactici* did not. The reduction of nitrate to nitrite by *L. plantarum* has been noted elsewhere (Costilow & Humphreys 1955; Langston & Bouma 1960; Rogosa 1961).

Little cured-meat pigment was produced when Lactacel was used to inoculate meat containing nitrate (Table 1). Lactacel DS resulted in the production of cured-meat colour due to the reduction of nitrate to nitrite by *L. plantarum.* Lactacel MC, which

TABLE 1

The production of cured-meat colour in a meat system by lactic acid starter cultures*

Starter culture	Absorbance (540 nm)† of extracts of meat containing:		
	$NaNO_3$	$NaNO_2$	nothing
Lactacel (*Pedicoccus acidilactici*)	0·018	0·165	0·020
Lactacel DS (*Lactobacillus plantarum*)	0·120	0·120	0·025
Lactacel MC (*Pediococcus acidilactici* and *Lactobacillus Plantarum*)	0·195	0·150	0·065

* Autoclaved beef which had been ground to a paste.
† The characteristic absorption peak of cured-meat colour.

contained both *L. plantarum* and *P. acidilactici*, also produced cured-meat colour. Good cured meat colour was obtained with all starter cultures when nitrite was present.

The authors express their thanks to Merck and Co., Rahway, N.J., for their generous gift of the Lactacel starter cultures.

References

ANON. 1957 *Manual of Microbiological Methods*. Committee on Bacteriological Technic, Society of American Bacteriologists, New York: McGraw-Hill Book Co.

ANON. 1973*a* Concentrated Lactacel DS for the production of fermented sausages. *Merck Food Products Application Bulletin* 203–257, Rahway, N.J.: Merck and Co., Inc.

ANON. 1973*b* Concentrated Lactacel MC for the production of fermented sausages. *Merck Food Products Application Bulletin* 203–256, Rahway, N.J.: Merck and Co., Inc.

COSTILOW, R. N. & HUMPHREYS, T. W. 1955 Nitrate reduction by certain strains of *Lactobacillus plantarum*. *Science, New York* **121,** 168.

DEIBEL, R. H. & EVANS, J. B. 1957 'Nitrite burn' in cured meat products—particularly in fermented sausages. *Bulletin* No. 32, Chicago: American Meat Institute Foundation.

HORSNEY, H. C. 1956 The colour of cooked cured pork. I. Estimation of the nitric oxide-haem pigments. *Journal of the Science of Food and Agriculture* **7,** 534–540.

LANGSTON, C. W. & BOUMA, C. 1960 A study of the microorganisms from grass silage. II. The lactobacilli. *Applied Microbiology* **8,** 223–234.

NIINIVAARA, F. P. 1955 Über den Einfluss von Bakterienreinkulturen auf die Reifung und Umrötung der Rohwurst. *Acta Agralia Fennica* **84,** 1–128.

ROGOSA, M. 1961 Experimental conditions for nitrate reduction by certain strains of the genus *Lactobacillus*. *Journal of General Microbiology* **24,** 401–408.

SHARPE, M. E., FRYER, T. F. & SMITH, D. G. 1966 Identification of the lactic acid bacteria. In *Identification Methods for Microbiologists* ed. Gibbs, B. M. & Skinner, F. A. Society for Applied Bacteriology, Technical Series No. 1. Part A, pp. 65–79. London and New York: Academic Press.

ZAIKA, L. L., ZELL, T. E., SMITH, J. L., PALUMBO, S. A. & KISSINGER, J. C. 1976 The role of nitrite and nitrate in Lebanon bologna, a fermented sausage. *Journal of Food Science* **41,** 1457–1460.